Bacterial Immunofluorescence protocol

(Adapted from Jose et al., 2005, doi: 10.1016/j.ab.2005.08.019)

1) For every cell type that needs testing, grow a culture of bacterial cells in 5mL LB (+antibiotics) overnight at 37 ˚C.
2) Next morning, take OD_{600} of the cultures (OD of 1 for *E. coli* corresponds to ~10^8 cells/mL), and dilute into 2 fresh 5mL LB tubes (+antibiotics) to OD_{600} of ~0.01. To one of these tubes, add IPTG to end concentration of 1mM. Incubate both tubes in a 37 ˚C shaker.
3) After ~3 hr of incubation, start monitoring OD of the cultures every half hour. We want to fix these cells at an OD_{600} of ~0.5.
4) As soon as a culture reaches OD_{600} of ~0.4-0.5, spin down 1mL of the culture in an Eppendorf tube at 8000xg (=rcf) for 1 min, and carefully discard the supernatant (be careful so as to only remove the supernatant, without disturbing the cells in the pellet).
5) Re-suspend the pellet in 1mL 1xPBS by pipetting up and down 5 times. Spin down the cells at 8000xg (=rcf) for 1 min, and carefully discard the supernatant.
6) Repeat the PBS wash in Step-5 two more times.
7) Now, re-suspend the cells in 0.5mL 1xPBS by pipetting. Add 0.8 uL BSA (NEB# B9000S, which is supplied with the restriction enzymes) to the re-suspended 1mL cells (end conc. ~3% BSA), and mix by pipetting.
8) Drop 50uL of the resuspension on a coverslip (round coverslips preferred), and incubate at 37 ˚C until it is completely dry. Once dry, save the coverslip at RT until all the cultures have been processed similarly.
9) Once all cultures have been dried onto coverslips, prepare petriplates (one for each coverslip) with a parafilm square in each plate.
10) Place the coverlips bacterial-side-up onto a parafilm square (1 coverslip per plate, label the petriplate- these plates can be re-used later). Move the plates to a fume hood, and add a few drops of 1xPBS+4%(para)formaldehyde onto the coveslip, enough to cover all area. Incubate at room temperature for 20 min. [FIXING STEP] **All handling of (para)formaldehyde must be done inside a fume hood, since it's quite toxic!**
11) Remove the fixing solution by pipetting off, and gently absorbing with a tissue paper.
12) Wash the slips by adding ~250-500uL 1xPBS, incubating at RT for 10min, then pipetting off/ absorbing. Do this 3 times.
13) Add 250-500uL blocking solution (1xPBS+3%BSA) onto the coverslip, and incubate at RT for 15 min. [BLOCKING STEP]
14) As before, wash the slips by adding ~250-500uL 1xPBS, incubating at RT for 10min, then pipetting off/ absorbing. Do this 3 times.
15) Add a blotting paper base to each petriplate, dampen the base by spraying/ sqirting some distilled water, and place a dry parafilm square on top. This keeps the setup moist and prevents evaporation during longer incubations. Put back the cover slips onto the parafilm square.
16) Add ~50uL diluted Primary Antibody (1˚Ab) (1xPBS+3%BSA+500x diluted Ab) onto the coverslip to cover the cells, and incubate on ice/ 4˚C for 1 hr. [PRIMARY INCUBATION STEP] The primary we'll use is ANTi-FLAG Sigma F1804 (mouse monoclonal IgG).
17) As before, wash the slips by adding ~250-500uL 1xPBS, incubating at RT for 10min, then pipetting off/ absorbing. Do this 3 times.
18) **This step onwards, everything must be done in the dark.** Add ~50uL diluted Secondary Antibody (2˚Ab) (1xPBS+3%BSA+500x diluted Ab) onto the coverslip to cover the cells, and

incubate on ice/ 4˚C for 1 hr. [SECONDARY INCUBATION STEP] The secondary we'll use is goat anti-mouse Alexa Fluor 488.

19) As before, wash the slips by adding ~250-500uL 1xPBS, incubating at RT for 10min, then pipetting off/ absorbing. Do this 3 times.
20) Add a drop of the mounting medium (ProLong Diamond Antifade Reagent, Fisher #15372192) on a glass slide and place the coverslip on top of it (bacterial-side-down).
21) Seal the edges of the cover-slip with nail-polish, and save in the fridge (4˚C), for later visualization.